Trends in Genetics

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Opinion

Rewards and dangers of regulatory innovation

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Adaptive evolution often involves structural variation affecting genes or cis-regulatory changes that engender novel and favorable gain-of-function gene regulation. Such mutation could result in a favorable dominant trait. At the same time, the gene product could be dosage sensitive if its change in concentration disrupts another trait. As a result, the mutant allele would display dosage-sensitive pleiotropy (DSP). By minimizing imbalance while conserving the favorable dominant effect, heterozygosity can increase fitness and result in heterosis. The properties of these alleles are consistent with evidence from multiple studies that indicate increased fitness of heterozygous regulatory mutations. DSP can help explain mysterious properties of heterosis as well as other effects of hybridization.

Highlights

Regulatory mutations affecting the expression level and pattern of dosage-sensitive genes can create dosage imbalance in cells affected by the expression change.

The deleterious effect of dosage imbalance is higher in homozygous and lower in heterozygous individuals.

Pleiotropism of regulatory mutations is possible. In addition to the dosage-sensitive trait, the mutation may engender a dominant advantageous trait.

Individuals that are heterozygous for such mutations will be fitter than the homozygotes.

Pleiotropism may help explain heterosis, the vigor displayed by hybrids. Hybrids are heterozygous at many loci, causing transgressive fitness compared with homozygous parents.

Pleiotropy of regulatory mutations

Organisms evolve through mutations that alter either gene products or their regulation, such as increasing or expanding expression. These gain-of-function changes can have remarkable consequences, such as engendering a new, advantageous trait (Figure 1, Key figure; Figure 2A). Additional effects are possible because proteins and RNAs can affect fitness in a dosage-sensitive manner. A dosage-sensitive protein (or RNA) is in balance with networked factors, and its changed expression may violate the optimal stoichiometric ratio in the affected cells. In the hypothetical example involving a herbivore (Figure 1), expanding the expression domain of a growth regulator lengthens multiple vertebrae. The resulting longer neck increases foraging efficiency. At the same time, the connected dosage imbalance may result in a different, disadvantageous trait such as a spindly and wobbly neck that reduces fitness through reduced speed and movement. The evolutionary success of this innovation will depend on the balance of the pleiotropic traits, which is in turn affected by the genotypic state. (See Box 1.)

Genetic behavior of dosage-sensitive pleiotropy

Dosage-sensitive **pleiotropy** (DSP; see Glossary) can develop when regulatory mutations occur in dosage-sensitive genes [2–4]. The altered expression of the gene product disrupts the stoichiometric balance with interacting factors compromising fitness of the mutant and subjecting the mutation to purifying selection [5,6]. If the same regulatory change has an independent beneficial effect, overall fitness may still exceed that of the wild type (Figure 2). DSP is a special case of the developmental pleiotropy observed for many genes [7–9]; the deleterious effect is a dosage-dependent consequence of **regulatory innovation**. If dosage imbalance decreases function of a protein, how can this type of allele exert dominance? Multiple dominant conditions affecting domesticated animals result from **haploinsufficiency** [10]. Furthermore, different quantitative or threshold responses to imbalance are possible for different traits controlled by the same gene [1,11]. Loss of fitness can occur even if there remains considerable activity of the dosage-sensitive protein. Therefore, current understanding of gene function and evolution argues that DSP is possible and that some genes can display **haploproficiency**. Importantly, heterozygosity reduces the deleterious, additive effect of a DSP allele while conserving any dominant effect. For this reason, DSP can help explain mysterious genetic phenomena such as transgressive performance of hybrids (**heterosis**) as well as hybrid impairment (Box 1).

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Key figure

Pleiotropic effect of regulatory mutation altering expression

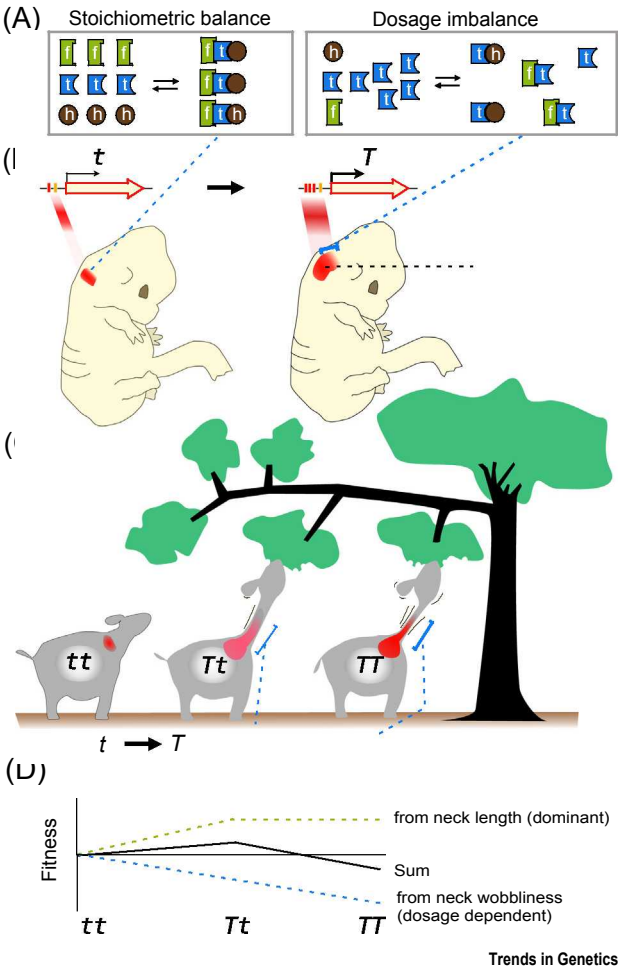

Figure 1. The example in a hypothetical herbivore employs a mutation affecting neck length. The concept is broadly applicable: The example could have been based on other systems and traits, such as root length (Figure 2A), or pigment development in plants. (A) Proteins f, t, and h form a trimeric complex. Left: Equal expression off, t, and h results in stoichiometric balance and optimal complex formation. Right: Excess of the bridge subunit t decreases the concentration of the trimeric complex (after [1]). (B) The t → T mutation in a hypothetical gene regulating vertebrae number and length causes an expansion in its expression pattern (red), resulting in neck lengthening. (C) Neck lengthening enables the animal to forage on previously inaccessible high leaves. This trait is dominant, being displayed equally by heterozygote and homozygote. At the same time, the neck is weakened in a dosage-sensitive mode because the regulatory protein is expressed in cells where it was not previously expressed, altering its stoichiometric ratio with cofactors. (D) The fitness consequences of this mutation will differ according to zygosity. TT homozygotes are most affected. For example, they display defects of the neck muscle, resulting in a wobbly neck. The affected animals are slower in their movements and therefore are less fit than the wild type. Tt heterozygotes have a thicker neck and display lower muscular malfunction than the TT homozygotes. On balance, the reduced muscular function in Tt individuals is offset by the foraging advantage and results in increased fitness of hybrids.

Molecular mechanisms of DSP formation

Regulatory mutations can act in trans or in cis. DSP can arise from cis-acting mutations in dosage-sensitive genes that expand or decrease gene activity (Figure 2). The simplest

Glossary

Cis-regulatory innovation: advantageous change in a cis-regulatory element of a gene. DNA elements in the promoter region of a gene exert a regulatory effect on the transcriptional unit residing on the same DNA molecule (i.e., in cis). Small to large changes in these elements, including deletion and insertion, can result in dramatic expression changes.

Copy number variation: change in copy number of gene elements, genes, and chromosome segments resulting from deletion, insertion, and duplication. These changes can affect gene expression and regulation and have frequent phenotypic effects.

Dosage sensitivity: property displayed by certain genes resulting in variable intensity of one or more traits, depending on gene copy number. Dosage sensitivity is exemplified by the dramatic effect of aneuploidy, the property of having more or less than the standard number of chromosomes.

Gene balance hypothesis: the theory that proper stoichiometry of gene products, protein, or RNA is needed for optimal cellular function.

Haploinsufficiency: a property of dosage-sensitive genes resulting in a heterozygous phenotype, which in some cases can be deleterious. From a genetic point of view, it may appear as incomplete dominance (homozygote displays a trait more intensely than the heterozygote) or dominance (heterozygote is affected, homozygote dies prematurely).

Haploproficiency: a property of dosage-sensitive genes resulting in an advantageous heterozygous phenotype.

Heterosis: enhanced fitness, growth, or productivity of the hybrid progeny when compared with either parent. This is also called 'best parent heterosis' and is consistent with the original observation of hybrid vigor. Sometimes, quantitative biologists and breeders use the term 'midparent heterosis' to refer to a quantitative trait that in the hybrid exceeds the mean of the parental values.

Pleiotropy: influence of a gene or alele on two or more apparently unrelated traits.

Structural variation: large-scale change (more than a few nucleotides) in DNA, such as insertion, deletion, duplication, inversion, and translocation.

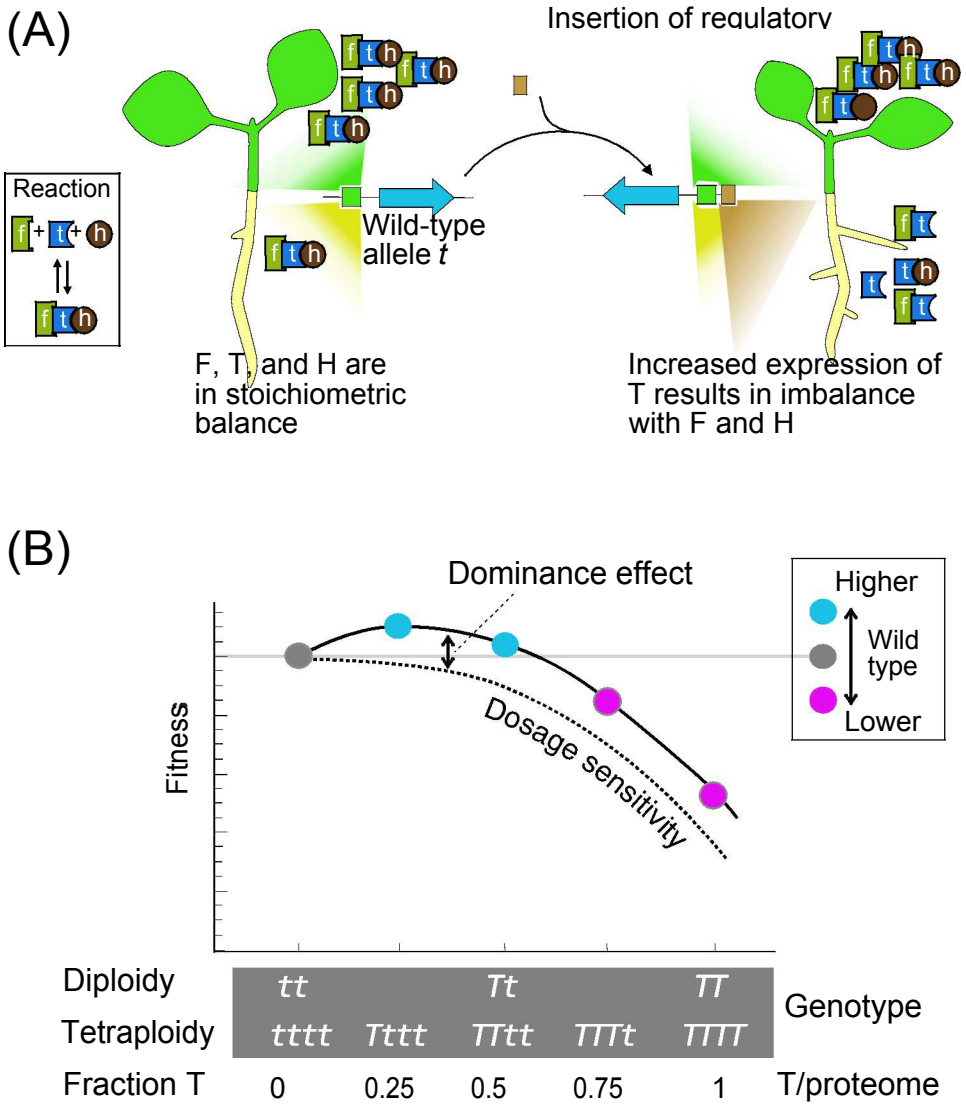

Figure 2. Nature and fitness effects from a dosage-sensitive pleiotropic allele (T). (A) A regulatory mutation increases expression of gene T in the root, causing localized imbalance. In this hypothetical situation, fitness is increased by the dominant effect of root branching and decreased by an additive effect that decreases growth. (B) Mutation of the ancestral t alele to T increases fitness in a dominant mode (not dosage sensitive) and decreases fitness according to a dosage-sensitive response. Totalfitness (circles) is the sum of the two effects. The favorable dominant effect increased fitness, regardless of dosage or ploidy. Factor T is expressed in a novel celular environment where it interacts with a naive proteome, which is in stoichiometric imbalance. The dosage sensitivity curve ilustrates the progressive loss of fitness. Total fitness is positive in the heterozygotes Tttt, TTtt, and Tt, which minimize the imbalance factor.

mechanism for regulatory change is duplication, a type of **structural variation** [12–14]. If the duplicated segment contains multiple genes, pleiotropy may result from the action of two or more genes. Alternatively, a promoter or enhancer cis-regulatory module that binds a regulator could be deleted or created. The frequent cis-evolution of promoters [15–18] is evident by comparison of orthologous gene regulation in related species and can be responsible for both additive and dominant traits [17,19], such as tolerance to a common stress [20–22]; novel coloration [16,23]; or a change in organ size, shape [24,25], and number [24,26,27].

Dosage sensitivity

An intuitive rationale for **dosage sensitivity** is cellular homeostasis: A metabolite or protein is needed in an optimal dose and less or more of it could be deleterious. Genes whose expression affects the concentration of the sensitive component display dosage sensitivity. Another, more elegant explanation for dosage sensitivity is the **gene balance hypothesis**, which states, 'The stoichiometry of members of multisubunit complexes can affect the amount of functional complete product, ... and ultimately, the phenotype and evolutionary fitness' [4]. Regardless of the underlying mechanism, dosage-sensitive genes are common. They encode transcription factors such as Gal4 [28], subunits of multiprotein complexes [2,29], or disordered proteins [3], but even genes encoding biosynthetic enzymes are coregulated in bacteria and yeast, indicating widespread efficiency of precise stoichiometry [30]. Chromosome copy number changes, aneuploidy, and gene **copy number variation** have far-ranging effects on phenotype [4,14]. In yeast, changes in the dosage of single genes are often deleterious, and, depending on the environmental conditions, dosage sensitivity can affect 5%–50% of all genes [2,3]. Systematic changes in expression of ~100 test genes revealed that 53% resulted in lower fitness upon low expression, whereas 30% decreased fitness upon high expression [6] (Figure 3). Interestingly, the environment affects the response; dosage sensitivity varies according to gene and conditions [6]. Some genes, such as TUB1 (Figure 3), display the classical 'rounded hill' response with the zenith at the wild-type expression level; responses, however, can be quite varied. In summary, there is compelling evidence for fitness changes, both positive and negative, as a consequence of regulatory mutations.

One expectation for homozygous DSP alleles is that hemizygosity should increase fitness or a fitness proxy such as growth. Interestingly, multiple studies have tested the effect of heterozygous deletions to identify haploinsufficient genes in budding and fission yeast and in human stem cells (Figure 4). In addition, these studies identified a substantial set of genes that increase fitness when a single allele is active and, therefore, are 'haploproficient' [31–34].

Evolutionary fate of DSP

Purifying selection should eliminate any allele that decreases fitness. Notwithstanding this expectation, such alleles exist [6]. A recent survey of ~18 000 natural, putative cis-regulatory sequences of yeast found that ~500 resulted in expression changes that compromised fitness, decreasing it by 1% or more [35]. Kremling et al. [5] reported comparable results for maize, stating that 'even intensive artificial selection is insufficient to purge genetic load.' Sharon et al. concluded that these

Box 1. DSP genes and deleterious hybrid effects

The potential for a buffered DSP locus (Figure 6A) to decrease fitness reemerges upon hybridization. In the absence of sex chromosomes, the F1s are likely to be viable and fit because all factors in a complex should be balanced. Dosage problems emerge in the F2, when independent assortment of the complex genes is unlikely to reproduce the balanced parental or F1 genotypes. This is consistent with the coadapted gene complexes theory underlying Dobzhanski-Muller-Bateson incompatibilities and resulting from diploid level interactions [57–59]. For three genes, the P of a balanced F2 = 15.5%. For four genes, P = 7%, and so forth. Large adapted complexes would result in widespread F2 inviability [60].

In hybrids where A or epistatic loci become selectively haploid, the imbalance appears in F1s (Figure 6). Haldane's rule (HR) states that lethality or sterility of hybrids wil preferentialy affect the heterogametic sex [61]. DSP aleles can account for HR in organisms that carry out sexual compensation by doubling expression of sex-linked genes in the heterogametic sex (Figure 'A). Whether the heterogametic sex hybrids are unviable or sterile may depend on the tissue affected by unbalanced regulation. Sex-linked DSP aleles that affect a change in an essential organ may result in lethality, whereas those that affect a change in reproductive cels may result in sterility. The model in Figure 'A is consistent with the rescue of heterogametic sex sterility by duplication of X in one parental species [62]. Furthermore, the dominance hypothesis for HR [42] can apply to DSP because dosage sensitivity of DSP alleles can satisfy the requirement for partial recessivity. On the other hand, DSP cannot easily account for HR in organisms that balance sex chromosome expression by chromosome inactivation (Figure B).

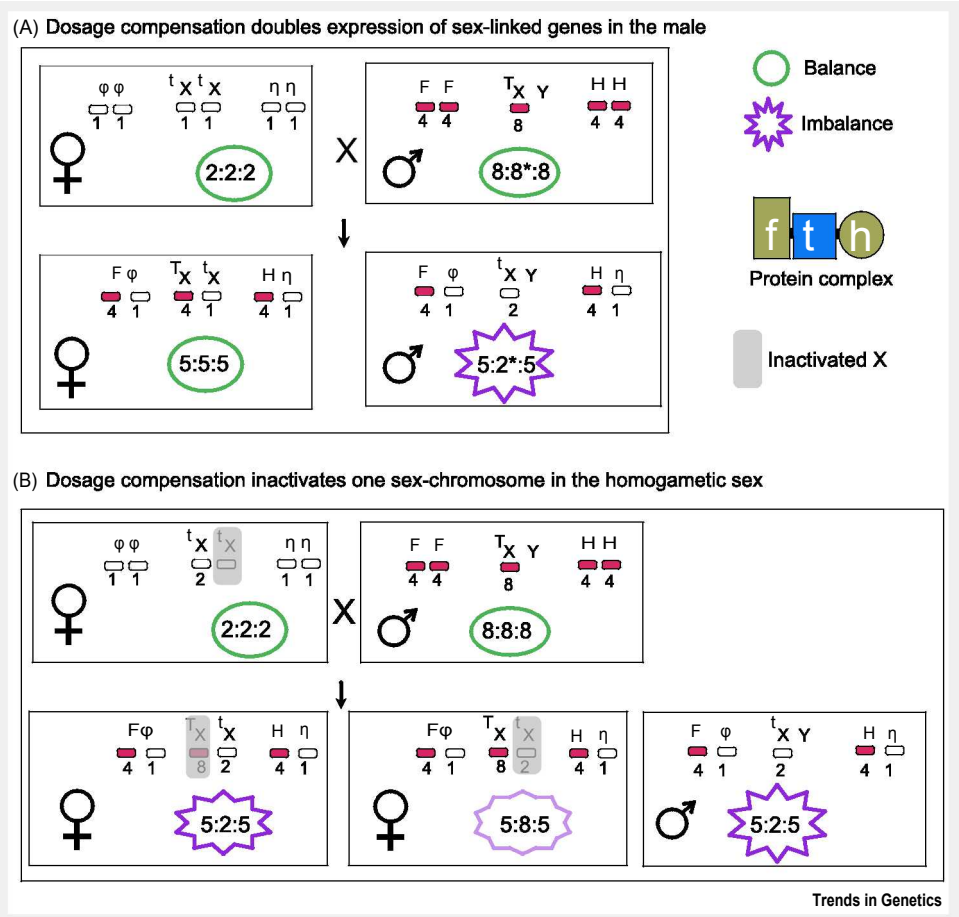

Figure I. DSP and the Haldane rule. Wide hybridization combines different coadapted orthologous genes from a 'red' XY male and a 'white' XX female. The DSP gene T is X-linked. T and t represent, respectively, the evolved and ancestral DSP aleles. (A) When dosage compensation works by doubling expression of sex-linked genes in the heterogametic sex, the F1 female produces the appropriate amount of the complex, but the male does not. This outcome is consistent with Haldane's rule. The reciprocal mating has a similar imbalance (not shown). (B) When dosage compensation works through inactivation of the sex chromosome in the homogametic sex, progeny of both sexes are imbalanced. If the 5:8:5 combination (female on the right side) is less severe, half of the females would be less affected. Consistent with this scenario, monosomy is typically more severe than trisomy.

alleles may also have unknown positive effects (i.e., are pleiotropic and thus fit my description of DSP) [35].

Population genetics theory predicts that novel DSP alleles can persist in a population as balanced polymorphisms (Figure 5), depending on allele effect and population size [36,37]. Figure 5 illustrates a classical simulation of this prediction: The wild-type t allele, when homozygous, confers a fitness of 0.9. The derived T allele starts arbitrarily at 1% frequency in a population of 10 000. A heterozygote advantage as small as 0.005 is sufficient to increase the allele frequency to 5%. The predicted frequency plateau depends on the fitness of the TT homozygote, reaching 0.5 when $Fitness_{\alpha\alpha} = Fitness_{AA}$. Even when the fitness penalty for TT individuals is strong, such as a decrease from 0.9 to 0.75, the T frequency can still increase. This is because the heterozygotes are favored and the TT homozygotes are unlikely when T frequency is low.

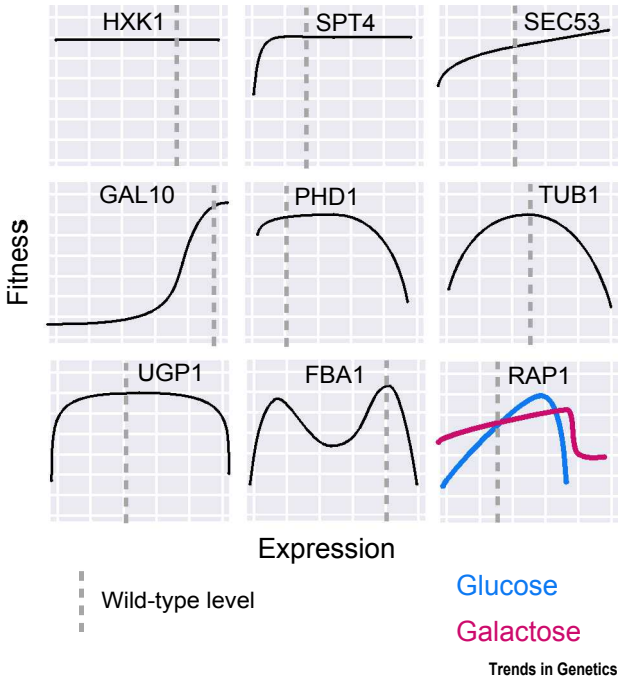

Figure 3. Variation among different genes in their fitness response to dosage. The fitness curves for nine genes derived by Keren et al. [6] are redrawn here. In the case of RAP1, the comparison of glucose versus galactose media exemplifies the distinct effect of environment on the dosage response.

Over time, the deleterious component of the T allele may be suppressed by epistatic beneficial mutations such that $Fitness_{tt} < Fitness_{tT} = Fitness_{TT}$. When loss of fitness is due to stoichiometric imbalance, these suppressors could restore stoichiometric equivalence of interacting gene products (Figure 6). Once buffered by these changes, allele Twill cease to be an active DSP allele, and, with the corollary loci, it will form a beneficial genotype. The resulting allele complex, compatible with effect size and recombination, should drive to fixation. Alternatively, drift could first fix Tin the population, setting up strong positive selection for compensatory mutations. These epistatic mutations can have a profound impact on the outcome of hybridization (Box 1).

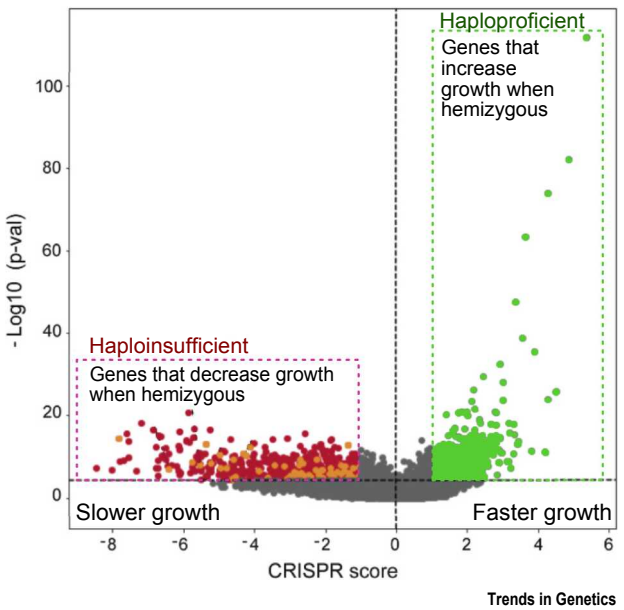

Figure 4. Effect of hemizygosity on growth in human stem cells. The figure was taken, with permission, from Sarel-Gallily et al. [34] and modified to highlight genes displaying haploproficiency. It describes the growth performance of CRISPR-edited, genome-wide mutants that are hemizygous for each individual gene. The CRISPR score equates to growth. The colored red box on the left highlights haploinsufficient genes (red: essential genes, orange: known haploinsufficiency genes). The green box contains haploproficient genes (i.e., genes that in hemizygous state increase growth).

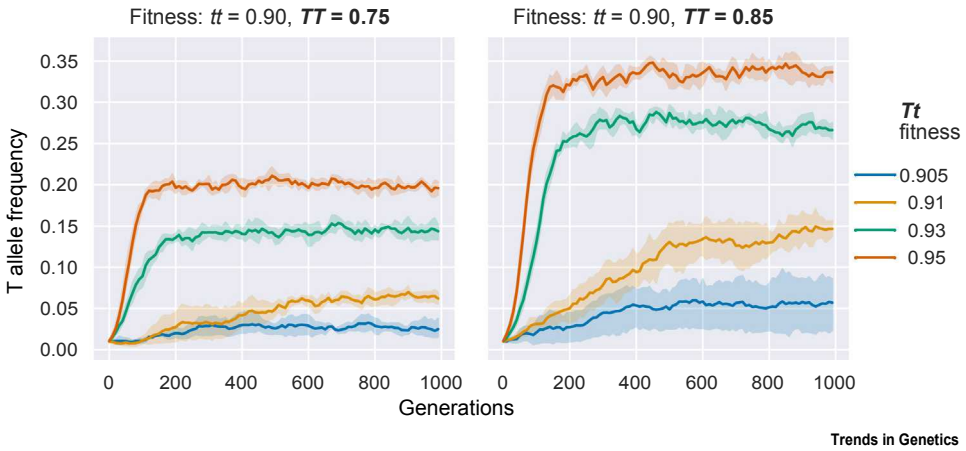

Figure 5. Frequency prediction for dosage-sensitive pleiotropic alleles. Simulation of allele frequency changes during 1000 generations of a diploid population in which dosage-sensitive pleiotropic alleles with different fitness properties start at 1% frequency. The simulation, a common tool in population biology courses, was carried out using the simuPop Population module [38] and was plotted using Seaborn [39]. Each line is the mean of five replicates. The shaded area surrounding each line represents the 95% confidence interval.

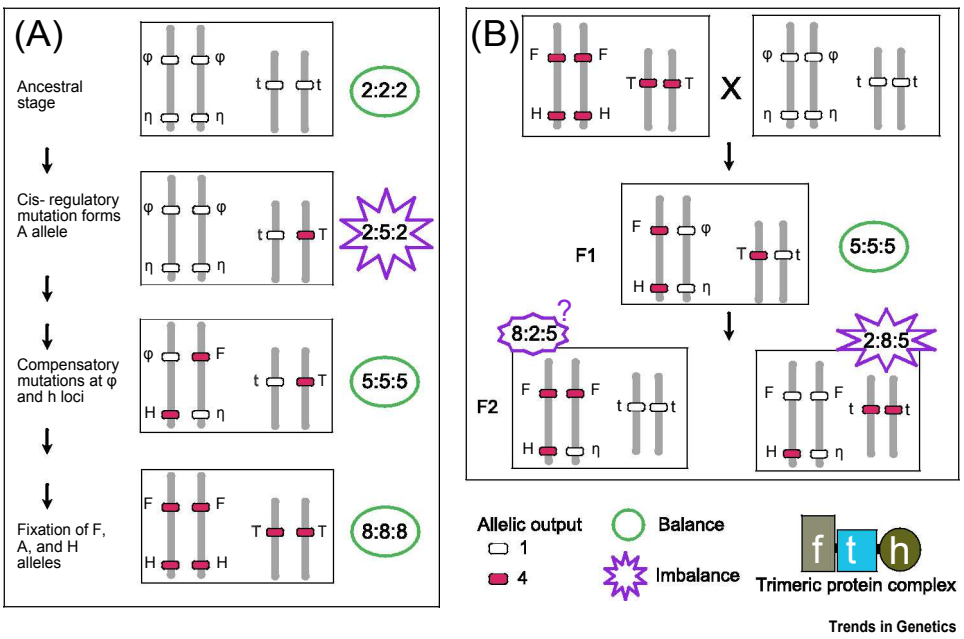

Figure 6. Evolutionary outlook for dosage sensitive pleiotropy. (A) Hypothetical evolution of a pleiotropic mutation affecting a three-gene system encoding dosage-sensitive subunits of a protein complex [4]. A mutation causing increased expression (t = 1 to T = 4) results in deleterious imbalance of a protein complex (legend at bottom right). If the mutation has a beneficial effect, it can persist (Figure 4). The deleterious effect of the T allele can be suppressed by compensatory mutations at interacting loci (φ = 1 - F = 4, η = 1 - H = 4) that increase the concentration of interacting protein subunits to regain a stoichiometric optimum. Eventually, the advantageous epistatic alleles become fixed. Sensitivity to epistatic interactions predicts that the effect of dosage-sensitive pleiot ropy loci could vary from species to species. (B) Wide hybridization combines different co adapted orthologous genes. The F1 progeny expresses balanced amounts of the protein complex subunits. Many F2s will express excess T subunits (e.g., bottom right genotype) and will be imbalanced. Some F2 will express excess of F and H subunits (e.g., bottom left genotype) and, depending on the biochemistry of the complex, may or may not display deleterious imbalance. Overall, the F2 fitness will be decreased.

DSP genes and heterosis

I define heterosis broadly as the transgressive performance of hybrids over parents, thus encompassing luxuriance and fitness. Alleles with DSP (Figure 1) can explain multiple properties of heterosis. In outcrossing large populations, fixation of DSP aleles is improbable until the TT genotypic burden (Figure 1) is aleviated by compensatory mutations. DSP can, however, be fixed by inbreeding and selection for DSP-derived dominant traits. Inbred individuals with different fixed DSP are likely to display good combining properties because their diverged genotypes maximize the chance of heterozygosity at DSP loci and thus heterotic gain. DSP can explain properties of heterosis that are difficult to explain with existing theories. The constant improvement of maize inbreds without erosion of heterotic gain [40] appears mysterious and is difficult to reconcile with the dominance hypothesis, the complementation of deleterious recessives [41]. Breeder's selection, however, is effective on deleterious alleles with large effects, but less so on DSP alleles because of their pleiotropism and relatively smal effects. Their persistence could maintain heterosis of suitable parental combinations. Furthermore, DSP loci can explain progressive heterosis, a phenomenon seen in polyploids, where four-way hybrids can exhibit higher heterosis than two-way hybrids [42–44], as well as the dependance on parental genome dosage in the heterosis of triploid hybrids [45]. In a four-way (four parents) tetraploid hybrid, dilution of each parental allele to one-fourth minimizes the negative dosage effect while maintaining beneficial dominant effects (Figure 1). At the same time, the increase in frequency of these alleles when inbreeding could explain rapid and progressive inbreeding depression (Figure 2). Indeed, small-effect dosage-sensitive loci that are purely deleterious could also explain both inbreeding depression and heterosis [42]. These loci may accumulate under special conditions, such as in low recombining regions of small populations [46].

Some yeast hybrids display heterosis, and the genetic resources available in this system should help determine the causal loci. Several investigations agree on the contribution of multiple small-effect genes but differ on the action mode of heterotic alleles: from underdominant [47] to overdominant and epistatic [48]. These discrepancies may derive in part from the genetics and environmental conditions of each experimental system. In one study, Herbst et al. [49] introduced genome-wide deletions of single genes (Δ) in Saccharomyces cerevisiae in heterotic hybrids of S. cerevisiae (c/c) × Saccharomyces paradoxus (p/p). They identified as heterotic genes whose deletion decreased growth of the hybrids (c/p > Δ/p). When these putative heterotic genes were tested in S. cerevisiae, however, hemizygosity (c/Δ) did not display dosage sensitivity, defying the expectation of dosage dependency. It may be premature, however, to take these results as proof that dosage-sensitive genes are not involved in heterosis. At least two considerations come to mind. First, the authors documented extensive remodeling of multiple regulatory pathways. Divergence between S. cerevisiae and S. paradoxus is considerable (5 My), and dissonance between the regulatory programs of the two species may complicate the comparison of dosage responses. Second, the study tested hemizygosity in parental and hybrid backgrounds under the reasonable assumptions that it would assess the heterotic potential of each locus. It is difficult, however, to determine whether loss of fitness in a hemizygous hybrid results from loss of a heterotic interaction or from simple haploinsufficiency. It may be more informative to compare the heterotic F1 hybrid with nearly isogenic hybrids where genome-wide replacement produced homozygosity at each tested locus (such as c/p versus c/c, c/p versus p/p). Single-gene analysis of putative heterotic loci has revealed surprising properties; the fitness effect of yeast ADH alleles and Neurospora sulfonamide resistance mutations are reminiscent of DSP [50,51]. How could the DSP hypothesis be further tested? Although most DSP are likely to have small effects and therefore be difficult to study individually, rare DSP with large effects are possible, and their characterization would help testing this proposal (see Outstanding questions).

The study of loci that may confer small fitness changes, although possible in yeast, is challenging in plants [6] and may be difficult to control. In maize, the heterotic effect of hemizygosity for

selected mutations [52] was attributed to pleiotropy, but these results have been disputed [53]. In tomato [54], and arabidopsis [55], however, large effects are evident for selected genes, although they may derive from physiological and developmental effects unrelated to DSP. Recent evidence in maize is encouraging. DSP alleles fit a maize model of heterosis based on the observation of rare, dosage-sensitive SNPs that are either deleterious or associated with variant expression. These alleles accounted for a decrease in seed size fitness [5,42,56]. The authors proposed that these alleles persist because they are in low recombination regions of the genome. Pleiotropism could also contribute to their persistence. In addition, because mutations that compensate for DSP emerge at other loci, relocation of these gene complexes to linked, nonrecombining regions would be advantageous to maintain the favorable interactions and the resulting fitness.

Concluding remarks

I propose that DSP alleles can emerge from adaptive evolution. They are characterized by the coincidence of two established but incompletely understood mechanisms: first, the deleterious consequences of copy number variation and aneuploidy, which can emanate from a single imbalanced gene product [2,4]; and second, the evolution of a beneficial trait through cis-regulatory changes [18]. Segmental duplications affecting multiple genes can easily result in pleiotropy and behave as a DSP allele. DSP could affect evolution, breeding, and human disease. Consistent with the proposal that nonadditive regulation of dosage-sensitive loci underlies heterosis [42], DSP alleles may make a significant contribution to hybrid success.

Outstanding questions

Unicellular organisms have provided fertile ground to study evolution, yielding results that in multiple cases support DSP. Can similar methods be applied to selected multicellular systems?

Could evolution through a specific DSP be demonstrated? An obvious challenge is that DSP genes are predicted to have small and difficult-to-measure effects. However, it may be possible to identify traits for which favorable and measurable changes may be acquired through a DSP.

Could DSP be engineered and tested experimentally? A large effect dosage-sensitive pleiotropic allele may be engineered by expressing a transgene encoding a predicted dosage-sensitive factor. A selected regulator may result in a useful dominant trait and enable measurement of the effect of zygosity on fitness.

Acknowledgments

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Declaration of interests

The author acknowledges no competing interests.

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